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2,4-Diamino-9*H*-pyrimido[4,5-*b*]indol-5-ols: Synthesis, in vitro cytotoxic activity, and QSAR investigations

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Abstract—A series of novel 2,4-diaminopyrimido[4,5-*b*]indol-6-ols has been synthesized and the in vitro cytotoxic activities were evaluated against four human cancer cell lines originating from solid tumors. An increase in activity was observed when a heteroaromatic ring was annulated on side *g* of the pyrimido[4,5-*b*]indole system to give compounds with activities comparable to ellipticine and cisplatin. To understand the experimental cytotoxic activities, QSAR investigations were performed, which showed a very good linearity between the experimental and predicted IC_{50}.

1. Introduction

Since the isolation of ellipticine (5,11-dimethyl-6*H*-pyrido[4,3-*b*]carbazole) (**1**) and the isomer olivacine (**3**) in 1959 from the leaves of *Ochrosia elliptica* (Apocynaceae), and the discovery of their antitumor activity,[1] a great number of derivatives and analogues have been prepared in order to enhance the anticancer activities, to improve the water solubility, and to reduce the toxicity. A new and promising candidate with a 9-hydroxy group is the olivacine derivative S 16020-2, which recently entered clinical trials.[2]

The preparation of 9-hydroxyellipticine derivatives such as elliptinium acetate (celliptium^{R}) (**4**), a clinical candidate on the market in France against advanced breast cancer, was mainly based on the hypothesis that 9-hydroxyellipticine (**2**), a metabolite of ellipticine (**1**), and other 9-hydroxyellipticine derivatives are metabolically oxidized in vivo to highly reactive 1,4-quinoneimine derivatives. These can react at the C-10 as electrophiles with bionucleophiles of the tumor cells; that is, amino acids, proteins, and hydroxyl groups of the DNA. A recent review[3] on ellipticine gives an excellent overview on the properties and multiple modes of antitumor activity by arylation (alkylation), intercalation with DNA, inhibition of DNA polymerases as well as interaction with topoisomerases I and II.

In the course of our synthetic studies with pteridine analogues,[4] we became interested in planar benzo- and heteroaromatic annulated indoles as DNA intercalators with potential antitumor activity; that is, analogues of 9-hydroxyellipticine (**2**), in which ring C is eliminated, giving tricyclic pyrido[4,3-*b*] indoles (γ-carbolines) with a 5-hydroxyindole substructure. This subunit might be oxidatively metabolized in the tumor cell to a 1,4-quinoneimine intermediate with possible antitumor activity. The synthesis of tricyclic analogues of ellipticine was first realized by Bisagni et al.[5] and led to pyrido[4,3-*b*]-indol-1-amines with interesting antitumor activities.

Retelliptine (**5**) (BD 84),[6] the 1-aminosubstituted ellipticine derivative, served as our starting point in the design of new analogues. The deletion of the benzene ring (ring C) gives 8-methoxysubstituted pyrido[4,3-*b*]indol-1-amines **6**, which have been already prepared by Bisagni et al.[5] In order to design new tricyclic analogues of 9-hydroxyellipticine (**2**), we envisioned an isosteric exchange of C-4 in **6** by a nitrogen atom to give the more

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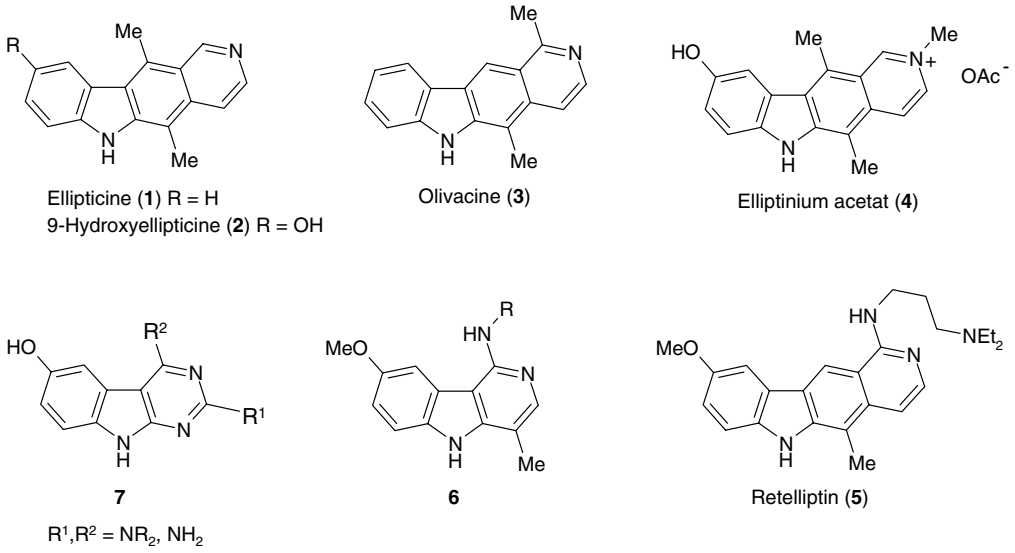


Figure 1. Structure of ellipticine and derivatives.

polar 2,4-diaminosubstituted pyrimido[4,5-*b*]indol-6-ols **7** as new tricyclic analogues of 9-hydroxyellipticine.

Recently, pyrimido[4,5-*b*]indol-4-amines and -2,4-diamines have been synthesized by multi-step procedures. These heterocyclics have been found to be inhibitors of protein kinases MKK4 and MKK7,[7] as inhibitors of epidermal growth factor receptor tyrosine kinase,[8] as new antiasthmatics (i.e., PNU-142731A[9]), and agents against neurodegenerative disorders.[10] These interesting biological properties motivated us to prepare 2,4-diaminopyrimido[4,5-*b*]indoles **7** with a 5-hydroxyindole subunit (Fig. 1).

The method of choice for access to 2,3-substituted 5-hydroxyindoles is the Nenitzescu reaction, which involves treatment of alkyl β-aminocrotonates or β-aminovinylketones with 1,4-benzoquinone and derivatives.[11] Based on retrosynthetic considerations and successful synthetic experiments, we recently developed a one-pot synthesis of the title compounds by an extension of the Nenitzescu reaction, utilizing pyrimidine-2,4,6-triamines as new enamine components.[12] This one-step reaction promised to be useful in the preparation of a wide variety of related heterocyclics suitable for structure–activity relationships (SAR).

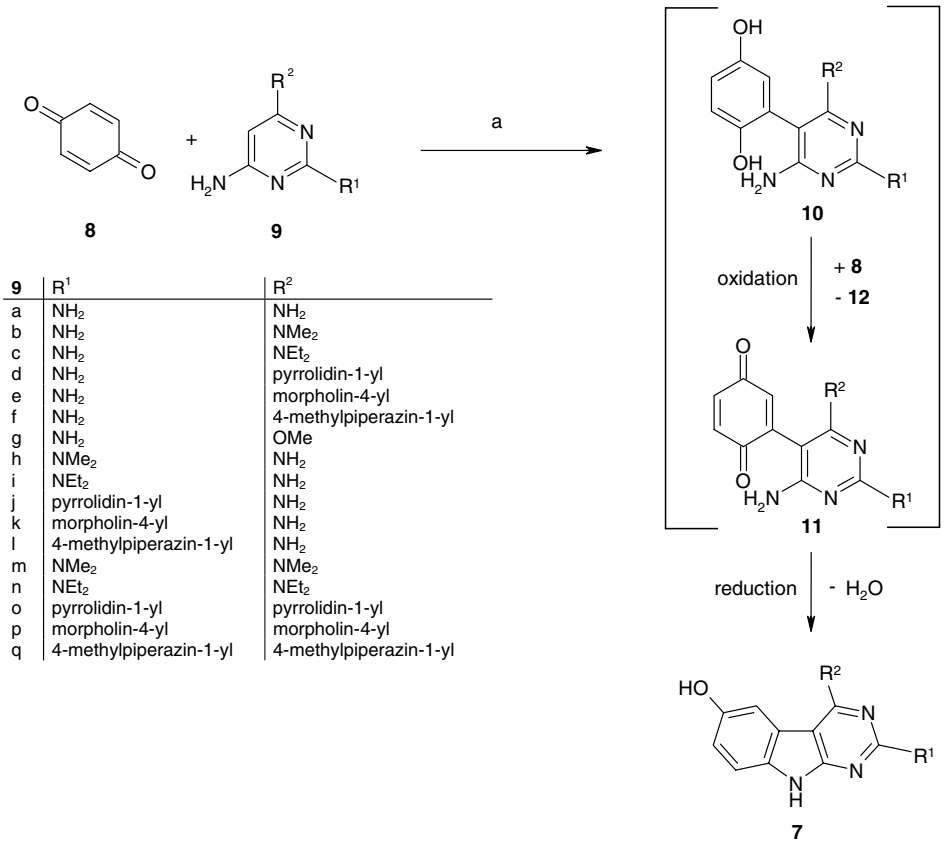
Herein we give a detailed report on the preparation and in vitro cytotoxic activity of a series of novel 2,4-diamino-9*H*-pyrimido[4,5-*b*]indol-6-ols **7** (R^1, $R^2 = NH_2$, NR_2) and tetracyclic congeners **14**, **16**, and **18**.

2. Results and discussion

2.1. Chemistry

The preparation of the title compounds **7** started with the synthesis of pyrimidine-2,4,6-triamines **9** according to literature procedures. All pyrimidines **9** possess a primary amino group in position 4 and primary or tertiary amino moieties in positions 2 and 6. One exception is **9g**, in which the amino group R^1 is exchanged by a methoxy function. In earlier semi-empirical MO calculations (AM 1 and PM 3) we have found that the ^{13}C NMR value of C-5 of pyrimidine-2,4,6-triamines **9** correlates well with charge density at C-5.[13] Therefore, the enamine reactivity of pyrimidine-2,4,6-triamines **9** toward 1,4-benzoquinone (**8**) and other 1,4-quinones can be calculated by the ^{13}C NMR value of C-5; a value of 75 ppm or lower will give a successful reaction. 2-Methoxypyrimidine-4,6-diamine, with a C-5 value of 78 ppm, showed no reaction with 1,4-benzoquinone (**8**). Glacial acetic acid is the solvent of choice for the preparation of the title compounds. Sometimes a mixture of EtOH and HOAc (50:1) was used. The amines **9** and 1,4-benzoquinone (**8**), at a molar ratio of 1:1.3, were each refluxed over 6 h in these solvents, typically giving dark residues after evaporation. Isolation and purification of compounds **7** were performed mainly by MPLC on silica gel (Scheme 1).

According to mechanistic studies of the Nenitzescu reaction,[11] the reaction sequence of our synthesis most likely begins with the addition of a pyrimidine-2,4,6-triamine **9** to the enone substructure of 1,4-benzoquinone (**8**), giving a Michael adduct **10**, which is oxidized by **8** to a quinone intermediate **11**. 1,4-Benzoquinone (**8**) is reduced in this process to hydroquinone (**12**). Following the intramolecular condensation of **11**, characterized by the concurrent elimination of water and reduction by the hydroquinone (**12**), the final 2,4-diamino-9*H*-pyrimido[4,5-*b*]indol-6-ols **7** are obtained in medium to low yields, which is typical for Nenitzescu reactions. In order to test our methodology as well as to explore the SAR of the title compounds, additional 1,4-quinones, example, bicyclic 1,4-quinones such as 1,4-naphthoquinone (**13**) were reacted with five selected pyrimidine-2,4,6-triamines, **9a**, **k**, **l**, **j** and **e**. These syntheses were achieved by refuxing the reactants in EtOH/HOAc (50:1) for



9	R^1	R^2
a	NH_2	NH_2
b	NH_2	NMe_2
c	NH_2	NEt_2
d	NH_2	pyrrolidin-1-yl
e	NH_2	morpholin-4-yl
f	NH_2	4-methylpiperazin-1-yl
g	NH_2	OMe
h	NMe_2	NH_2
i	NEt_2	NH_2
j	pyrrolidin-1-yl	NH_2
k	morpholin-4-yl	NH_2
l	4-methylpiperazin-1-yl	NH_2
m	NMe_2	NMe_2
n	NEt_2	NEt_2
o	pyrrolidin-1-yl	pyrrolidin-1-yl
p	morpholin-4-yl	morpholin-4-yl
q	4-methylpiperazin-1-yl	4-methylpiperazin-1-yl

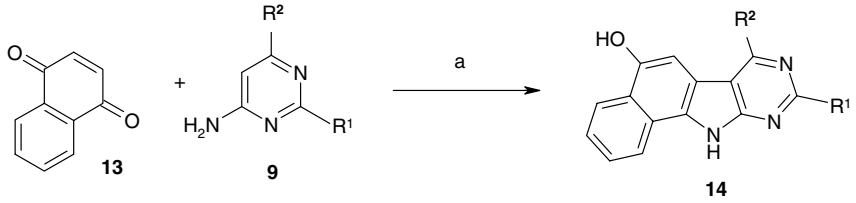
Scheme 1. Reagent and condition: (a) HOAc, reflux 6 h.

6 h, giving the tetracyclic benzo[*g*] annulated pyrimido[4,5-*b*]indol-6-ols **14a**–**e** in medium yields (Scheme 2).

Finally the *N*-heteroaromatic quinones quinoline-5,8-dione (**15**)[14] and quinoxaline-5,8-dione (**17**)[15] were treated with pyrimidine **9j** in boiling HOAc, yielding the tetracyclic systems **16** and **18**, which possess a 5-hydroxyindole subunit. Compounds **16** and **18** are structures that contain hitherto unknown heterocyclic ring systems (Scheme 3).

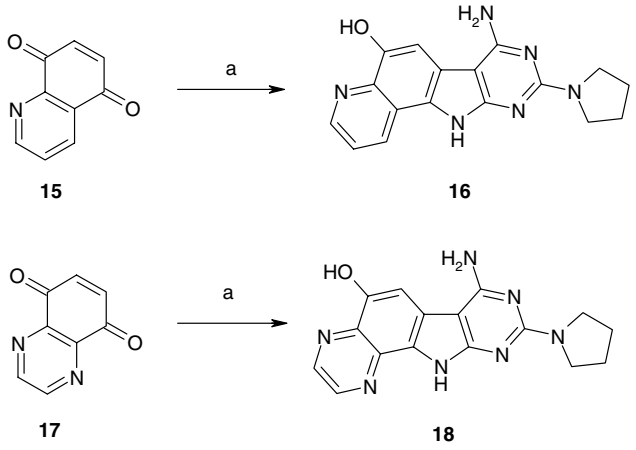
In order to study the influence of the hydroxyl group in position 6 of this new class of compounds on biological activity, we first synthesized the 6-methoxypyrimido[4,5-*b*]indol-2,4-diamine **19** by methylation of **7j** with methyliodide/NaH. The pyrimido[4,5-*b*]indole **22**, which lacks a hydroxyl group, was prepared by reacting 2-chlorocyclohexanone (**20**) with 2-pyrrolidin-1-ylpyrimidine-2,4-diamine (**9j**) in *n*-BuOH (under reflux), giving rise to the tetrahydroindole derivative **21**, which was finally oxidized by treatment with Pd/C in boiling xylene to yield 9*H*-pyrimido[4,5-b]indol-2,4-diamine **22** (Scheme 4).

To investigate the importance of an amine group in position 2 on the biological activity, the 2-unsubstituted 4-amino- and 4-phenylsubstituted pyrimido[4,5-*b*]indol-6-ols **24** and **26** were also prepared. Ring closure

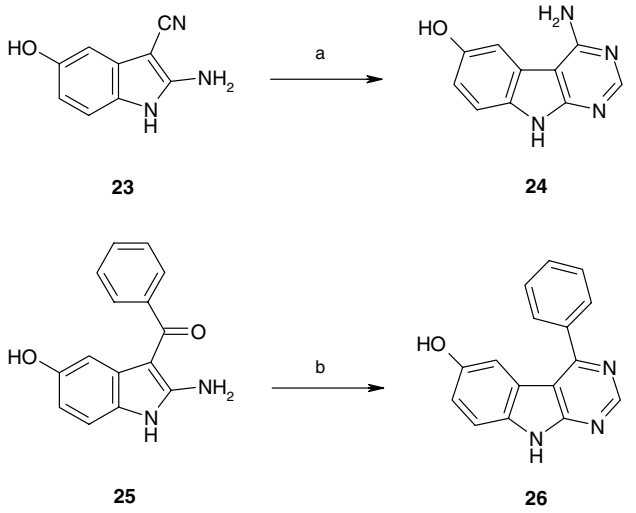


14	R^1	R^2
a	NH_2	NH_2
b	morpholin-4-yl	NH_2
c	4-methylpiperazin-1-yl	NH_2
d	pyrrolidin-1-yl	NH_2
e	NH_2	morpholin-4-yl

Scheme 2. Reagents and condition: (a) EtOH/HOAc (50:1), reflux 6 h.



Scheme 3. Reagents and conditions: (a) 2-pyrrolidin-1-ylpyrimidine-4,6-diamine (**9j**), EtOH/HOAc, reflux 6 h.



Scheme 5. Reagents and conditions: (a) formamide, dimethylformamide, formic acid (85%), reflux, 2 h; (b) formamide, formic acid (85%), reflux, 30 min.

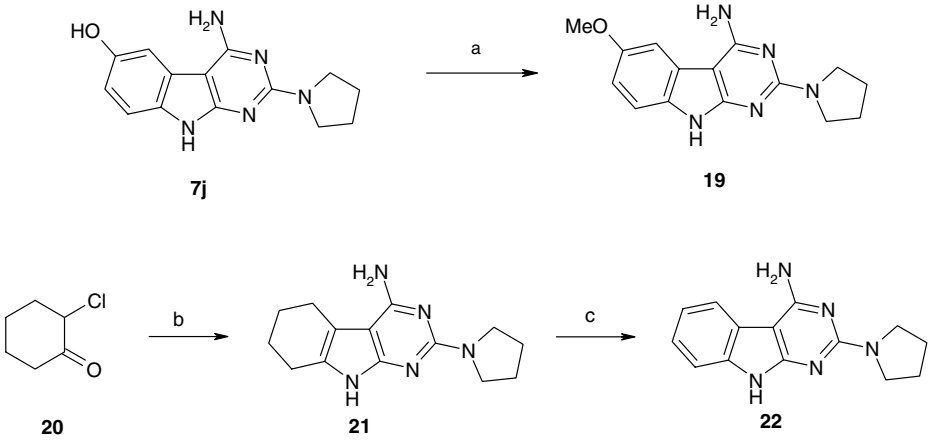
reactions of 2-amino-5-hydroxy-1*H*-indol-3-carbonitrile (**23**)[16] and (2-amino-5-hydroxy-1*H*-indol-3-yl)(phenyl)methanone (**25**)[16] with formamide under slightly varying conditions gave **24** and **26**, respectively, in modest yields (Scheme 5).

2.2. In vitro cytotoxic activity

To evaluate the in vitro cytotoxic potency of 2,4-diamino-9*H*-pyrimido[4,5-*b*]indoles **7a**–**q**, **14a**–**e**, **16**, **18**, **19**, **22**, **24**, and **26**, an established microtiter assay, based on cell staining with crystal violet, was used to measure the inhibition of cell growth caused by the test compounds. In these studies, four human cancer cell lines from solid tumors were used: two human bladder cancer cell lines 5637 (ACC 35) and RT-4 (ACC 412), and two human lung cancer cell lines A-427 (ACC 234) and LCLC-103H (ACC 384). The microtiter assay measures the degree of cell growth inhibition over a 4 d continuous exposure to drug; at the end of the drug exposure the adherent cells are stained with crystal violet. After washing out the non-bound dye, the cell-bound dye is redissolved in 70% ethanol/water and the optical density (OD) of the wells at $\lambda = 570$ nm is measured with a plate reader.[17] The OD is directly proportional to the number of cells in the well. The assay has been previously validated with the A-427 cell line by comparing the IC_{50} values obtained for cisplatin either by direct cell counting or by the microtiter assay.[18]

The results of the cytotoxic studies are reported in Table 1 and indicate pronounced SAR with regard to the cell growth inhibitory activity of the diverse compounds. In general, compounds that showed poor activity in one cell line were poorly active in the other three, and vice versa for compounds showing good activity. The differences in potency were all within a factor of five across the four cell lines. This was also the case with ellipticine and cisplatin, two compounds with known antitumor activity.

The type and substitution position of the amine substituents in compounds **7** had a noticeable effect on



Scheme 4. Reagents and conditions: (a) NaH/methyliodide/acetone, 12 h; (b) **9j**, *n*-BuOH reflux 6 h; (c) Pd/C, xylene, reflux 8 h.

Table 1. Cell growth inhibitory activity (IC_{50}, μM) of compounds **7a–q**, **14a–e**, **16**, **18**, **19**, **22**, **24**, and **26** against four human cancer cell lines

Compound	IC_{50} (μM) ±SD				
	5637	RT 4	A-427	LCLC-103 H	Average[b]
7a	20.5 ± 6.7	>20	20.5 ± 3.9	>20	20.5
7b[a]	>20	>20	>20	>20	
7c	14.0 ± 4.2	13.8 ± 11.4	8.94 ± 1.35	16.9 ± 4.0	13.41
7d	8.4 ± 2.2	13.6 ± 4.2	11.0 ± 1.1	17.6 ± 1.6	12.65
7e	15.3 ± 2.5	>20	13.7 ± 1.0	>20	15.30
7f	20.4 ± 5.3	>20	>20	>20	20.40
7g[a]	5.84	>20	8.02	10.7	
7h	15.4 ± 6.0	17.0 ± 13.0	21.6 ± 6.7	17.8 ± 6.1	17.95
7i[a]	>20	>20	>20	>20	
7j	16.9 ± 6.4	3.88 ± 1.56	16.8 ± 4.1	>20	12.52
7k	16.6 ± 2.7	21.7 ± 2.7	18.3 ± 1.7	>20	18.87
7l[a]	>20	>20	>20	>20	
7m[a]	>20	>20	>20	>20	
7n	4.49 ± 1.49	4.68 ± 1.99	2.39 ± 0.26	7.74 ± 1.83	4.83
7o	11.4 ± 2.6	8.58 ± 1.34	7.77 ± 1.84	13.9 ± 1.4	10.41
7p	9.96 ± 2.39	9.05 ± 0.26	5.02 ± 0.97	5.05 ± 1.28	7.27
7q	9.50 ± 1.87	3.83 ± 0.70	4.14 ± 1.02	12.6 ± 0.7	7.52
14a	2.39 ± 0.17	1.17 ± 0.44	3.51 ± 0.39	5.51 ± 0.65	3.15
14b	5.63 ± 1.66	3.69 ± 1.25	5.28 ± 0.43	4.54 ± 0.60	4.79
14c	0.77 ± 0.35	3.96 ± 1.38	2.85 ± 1.06	3.00 ± 0.91	2.65
14d[a]	>20	>20	>20	>20	
14e	2.18 ± 0.57	6.45 ± 2.59	4.19 ± 0.29	4.16 ± 0.52	4.25
16	0.56 ± 0.39	0.90 ± 0.14	1.97 ± 0.29	2.50 ± 0.36	1.48
18	2.51 ± 0.72	0.50 ± 0.23	3.18 ± 0.49	3.08 ± 0.69	2.32
19	15.0 ± 3.7	>20	15.3 ± 1.5	>20	15.15
22[a]	>20	>20	>20	>20	
24	10.0 ± 3.4	18.4 ± 2.9	4.79 ± 0.09	19.8 ± 5.6	13.25
26	2.24 ± 0.95	8.28 ± 3.59	1.36 ± 0.45	5.81 ± 0.88	4.42
Ellipticine	0.89 ± 0.20	1.21 ± 0.16	0.89 ± 0.06	0.80 ± 0.04	0.95
Cisplatin	0.37 ± 0.08	1.77 ± 0.37	1.27 ± 0.25	1.09 ± 0.40	1.13

Values with standard deviations (SD) are averages of at least three independent determinations. Values without SD for **7g** are averages of two determinations. Values >20 μM indicate less than 50% growth inhibition at 20 μM.

[a] Not used in the QSAR.

[b] Average IC_{50} used in the QSAR.

activity. The lead compound **7a** with 2,4-diamino groups was one of the weakest compounds tested. Generally, substitution of dialkylamines or heterocyclic amines at both position 2 and 4 increased activity compared to the analogues with just one of the positions substituted with an amine (i.e., compare **7p** with **7e** and **7k** or **7o** with **7d** and **7j** or **7q** with **7f** and **7l**). However, this trend was not dramatic and not always observed in all cell lines.

The poor water solubility of the parent compound **7a** prompted us to introduce basic piperazine rings into the aromatic heterocyclic ring system in order to facilitate salt formation. Compounds **7f** and **7l** with just one *N*-methylpiperazine ring were inactive in our cytotoxicity screen. However, the derivative with two *N*-methylpiperazine rings, **7q**, shows modest activity compared to the most active compound, **16**. In the group of tetracyclic compounds **14**, one of the most active compounds, **14c**, possesses a basic *N*-methylpiperazine. In fact, the basic nitrogen appears to directly improve activity because the morpholino derivative **14b** was less active. Thus, the introduction of piperazine rings as basic substituents offers a useful method to improve on the pharmaceutical properties of these compounds.

The absence of a free hydroxy group in position 6 did not completely eliminate activity; the methoxy derivative **19** retained some weak activity in the 5637 and A-427 cell lines (compare **7j** with **19**) but lost activity in the RT-4 cell line. However, analogue **22**, which lacks altogether an oxygen in position 6, was inactive. This is in contrast to ellipticine, which also lacks a hydroxy group but is quite potent, presumably because it is oxidized to 9-hydroxyellipticine in the cell.

A general increase in activity was observed when the phenol ring of **7** is annulated with an additional ring to give either naphthalene (**14**), quinoline (**16**) or quinoxaline (**18**) systems. Although they are connected differently, four annulated rings are also present in the structures of the ellipticines. The compound with the best overall activity in this series was that with the quinoline ring (**16**), possessing potency comparable to ellipticine and cisplatin (Table 1). Nevertheless, not all compounds in this series had good activity; that is, replacing the quinoline nitrogen with carbon distinguished the most potent compound **16** from one of the least potent **14d**. This is a remarkable SAR, indicating that compound **16** probably interacts with a specific target molecule to inhibit cell growth.

2.3. QSAR investigations

In order to understand the experimental biological data on a theoretical basis, we established a quantitative structure–activity relationship (QSAR) between the in vitro cytotoxic potency of 19 selected compounds and descriptors coding for atomic, fragment, and molecular properties of the molecules under consideration. The parameter of biological activity was the average IC_{50} value over all four cell lines. Compounds **7b**, **7i**, **7l**, **7m**, **14d**, and **22** were omitted due to lack of activity (IC_{50} values larger than 20 μM for all cell lines), compound **7g** due to the missing N4 substituent. Calculated molecular descriptors (i.e., mass, surface area, volume, molar refractivity, heat of formation, lipophilicity, lipole, HOMO and LUMO energies, moment of inertia, dipole moment, and Verloop sterical parameters), indices (e.g., connectivity, topology, and shape), group counts (i.e., atoms, rings, and functional groups) as well as the calculated partial charges on various atoms were included in the QSAR analysis.

The final model, generated by a multiple linear regression, comprises the following atomic and molecular descriptors: heat of formation (X1), LUMO (X2) and HOMO (X3) energies, Kier ChiV3 index (X4), and partial atomic charges on atoms N1 (X5), N2 (X6), N3 (X7), N4 (X8), $H1^+$ (X9), and O (X10). Figure 2 shows on the common compound scaffold the position of the atoms (indicated bold) used in the QSAR analysis.

The regression equation for the original data without standardization is given in Eq. 1.

$$\begin{aligned}\log 1/IC_{50} = {} & 0.008311389 \times X1 - 1.5044706 \times X2 \\ & + 1.3994405 \times X3 - 1.9093369 \times X4 \\ & - 5.8294468 \times X5 + 0.90101105 \\ & \times X6 + 3.9894164 \times X7 \\ & + 0.32782158 \times X8 - 13.69002 \times X9 \\ & - 2.9178424 \times X10 + 18.34861 \qquad (1)\end{aligned}$$

Figure 3 shows a plot of the predicted versus the experimental log 1/average IC_{50} values of the 19 selected active compounds (i.e., average $IC_{50} < 20$ μM) calculated by the final model given in Eq. 1.

Encouraged by the quality of the activity prediction ($r^2 = 0.95$, cross-validated regression coefficient $r^2_{CV} = 0.69$) obtained by using mainly quantum-mechanically derived descriptors, we selected the experimentally known compounds **24** and **26** as test cases for our method. However, both test molecules lack a substituent including N2, while compound **26** additionally including N4 (see Scheme 5); therefore we first had to rebuild the model without the descriptor for the partial atomic charge on the atoms N2 (X6) and N4 (X8). Nevertheless, diminishing the number of descriptors by one ($r^2 = 0.92$) or two ($r^2 = 0.89$) only slightly reduced the quality of the model. Applying the modified equations to the test compounds gives the following log $1/IC_{50}$ values (experimental average values over the four cell lines are given in brackets): **24**: 5.04 (exp. 4.88); **26**: 5.26 (exp. 5.35).

The predicted activity data compare well with the experimental values in terms of absolute values and ranking. The results show that the descriptors chosen are well suited to reproduce the complex and unspecific experimental data.

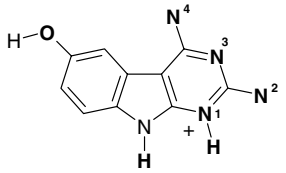


Figure 2. Common compound scaffold showing the position of the atoms (indicated bold) used in the QSAR analysis.

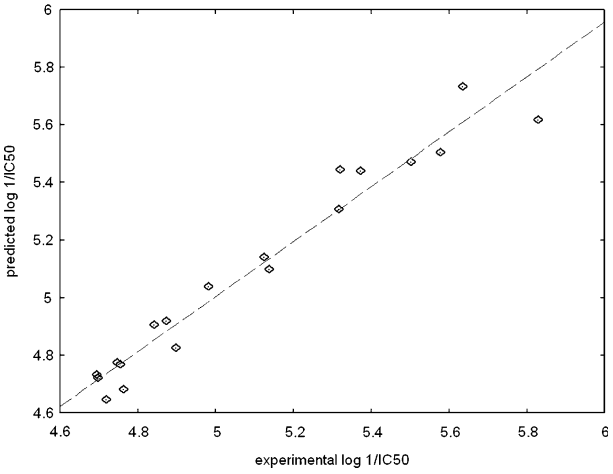


Figure 3. Average experimental IC_{50} versus calculated IC_{50} values for 19 compounds.

3. Conclusions

In this study, a series of 2,4-diaminopyrimido[4,5-*b*]indol-6-ols have been synthesized as analogues of 9-hydroxyellipticine and their in vitro cytotoxic activities were evaluated against four human cancer cell lines. An increase in activity was observed when a heteroaromatic ring was annulated on side *g* of the tricyclic ring system giving compounds **16** and **18**, whose cell growth inhibitory activity is comparable to ellipticine or cisplatin. Another useful modification was the introduction of a basic *N*-methylpiperazine ring as a salt-forming moiety; one such compound, **14c**, retained good cytotoxic activity. To help understand the cytotoxic potencies of this series of compounds, QSAR investigations were performed. The results showed a very good linearity between the experimental and predicted IC_{50}. Future work will aim to understand interactions of these compounds with molecular targets such as DNA as well as the mechanism(s) of cell death.

4. Experimental

4.1. General

Starting materials were obtained from commercial sources and were used without further purification. Solvents were dried by standard procedures. Reaction progress was observed by thin-layer chromatography making use of commercial silica gel plates (Merck, silica gel F_{254} on aluminum sheets). Column chromatography was done on silica gel 60 (Merck). Melting points were determined in open capillary tubes on a Buechi 510 melting point apparatus and are uncorrected. Elemental analyses were performed by the Institut für Organische Chemie (University of Erlangen/Nuremberg) using Carlo Erba Elemental Analyzer 1108. They are within ±0.4% of the theoretical values if not noted otherwise. ^{1}H nuclear magnetic resonance (^{1}H NMR) spectra were determined with a Bruker AM 360 (360 MHz) spectrometer in appropriate deuterated solvents and are expressed in parts per million (δ, ppm) downfield from tetramethylsilane (internal standard). NMR data are given as multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet), coupling constants (J), and number of protons. Mass spectra (MS) were taken with a Finnigan MAT TSQ 70 mass spectrometer in the electron impact mode (70 eV). Significant infrared (IR) spectra were obtained on a Jasco FT/IR 410 or Perkin-Elmer 1740 spectrometer.

Cell lines were obtained from the German Collection of Microorganisms and Cell Culture (DSMZ, Braunschweig, FRG) and were grown in RPMI culture medium supplemented with 10% fetal calf serum. Ellipticine and all reagents for the cell experiments were obtained from Sigma–Aldrich (Taufkirchen, FRG). Cisplatin was from ChemPur (Karlsruhe, FRG). Plastic cell culture materials were from Sarstedt (Nümbrecht, FRG). The optical density of crystal violet was measured with an Anthos 2010 plate reader equipped with a 570 nm filter (Salzburg, Austria).

4.1.1. General procedure for preparation of 9*H*-pyrimido[4,5-*b*]indol-6-ols 7, 14 and compounds 16 and 18. A mixture of the pyrimidine-2,4,6-triamine **9** (3 mmol) and either 1,4-benzoquinone (**8**) (3.6 mmol) or 1,4-naphthoquinone (**19**) (3.6 mmol) or quinoline-5,8-dione (**15**) (3.6 mmol) or quinoxaline-5,8-dione (**17**) (3.6 mmol) was refluxed for 6 h in either solvent A or B. The solvent was distilled off in vacuo and the dark residue was purified by MPLC on silica gel. Solvent A: HOAc, Solvent B: EtOH/HOAc = 50:1; eluent A: $CHCl_3$/MeOH = 95:5.

4.1.2. 2,4-Diamino-9*H*-pyrimido[4,5-*b*]indol-6-ol (7a). Preparation according to literature.[12]

4.1.3. 2-Amino-4-dimethylamino-9*H*-pyrimido[4,5-*b*]indol-6-ol (7b). Solvent A; eluent A, 238 mg, 32%, gray powder, mp 294–298 °C. IR (KBr): ν (cm^{-1}) = 2919, 1577, 1475, 1394, 1041. ^{1}H NMR: (DMSO-d_6); δ = 3.09 (s, 6H, NMe_2), 5.99 (s, 2H, exchangeable with D_2O, NH_2), 6.63 (dd, 1H, J_1 = 2.1 Hz, J_2 = 8.5 Hz, H-7), 7.05 (d, 1 H, J = 2.1 Hz, H-5), 7.07 (d, 1H, J = 8.5 Hz, H-8), 8.81 (s, 1H, exchangeable with D_2O, OH), 10.90 (s, 1H, exchangeable with D_2O, NH). MS m/z 243 [M$^+$]. Anal. Calcd for $C_{12}H_{13}N_5O$ (243.27): C, 59.25; H, 5.39; N, 28.79. Found: C, 58.97; H, 5.67; N, 28.82.

4.1.4. 2-Amino-4-diethylamino-9*H*-pyrimido[4,5-*b*]indol-6-ol (7c). Solvent A; eluent A, 471 mg, 58%, gray powder, mp >300 °C. The residue was crystallized from EtOH after cooling and evaporation. IR (KBr): ν (cm^{-1}) = 2969, 1592, 1554, 1383, 796. ^{1}H NMR: (DMSO-d_6); δ = 1.18 (t, 6H, –CH_2–CH_3, J = 7.1 Hz), 3.59 (q, 4H, 3.09, –CH_2–CH_3, J = 7.1 Hz), 5.96 (s, 2H, NH_2, exchangeable with D_2O), 6.63 (dd, 1H, H-7, J_1 = 8.5 Hz, J_2 = 2.1 Hz), 7.05 (d, 1H, H-8, J = 8.5 Hz), 6.93 (d, 1H, H-5, J = 2.1 Hz), 8.83 (s, 1H, OH, exchangeable with D_2O), 10.90 (s, 1H, NH, exchangeable with D_2O). MS m/z 271 [M$^+$]. Anal. Calcd for $C_{14}H_{17}N_5O$ (271.32): C, 61.98; H, 6.32; N, 25.81. Found: C, 61.79; H, 6.29; N, 25.89.

4.1.5. 2-Amino-4-pyrrolidin-1-yl-9*H*-pyrimido[4,5-*b*]indol-6-ol (7d). Solvent A; eluent A, 95:5; 158 mg, 25%, gray powder, mp >300 °C. IR (KBr): ν (cm^{-1}) = 2921, 1660, 1593, 1552, 1454, 1015, 772. ^{1}H NMR: (DMSO-d_6); δ = 1.90 (m, 4H, H-3′, H-4′), 3.76 (m, 4H, H-2′, H-5′), 5.81 (s, 2H, NH_2, exchangeable with D_2O), 6.58 (dd, 1H, H-7, J_1 = 8.5 Hz, J_2 = 2.1 Hz), 7.02 (d, 1H, H-8, J = 8.5 Hz), 7.25 (d, 1H, H-5, J = 2.1 Hz), 8.70 (s, 1H, OH, exchangeable with D_2O), 10.84 (s, 1H, NH, exchangeable with D_2O), MS m/z 269 [M$^+$]. Anal. Calcd for $C_{14}H_{15}N_5O$ (269.31): C, 62.44; H, 5.61; N, 26.00. Found: C, 62.14; H, 5.76; N, 26.23.

4.1.6. 2-Amino-4-morpholin-4-yl-9*H*-pyrimido[4,5-*b*]indol-6-ol (7e). Solvent A; eluent A, 273 mg, 32%, green powder, mp >300 °C. IR (KBr): ν (cm^{-1}) = 2965, 2851, 1604, 1561, 1439, 1200, 1110, 880. ^{1}H NMR: (DMSO-d_6); δ = 3.43–3.83 (m, 8H, H-2′, H-3′, H-5′, H-6′), 6.56 (s, 2H, NH_2, exchangeable with D_2O), 6.16 (dd, 1H, H-7, J_1 = 8.5 Hz, J_2 = 2.5 Hz), 6.84 (d, 1H, H-5, J = 2.5 Hz), 7.08 (d, 1H, H-8, J = 8.5 Hz), 8.90 (s, 1H, OH, exchangeable with D_2O), 10.89 (s, 1H, NH, exchangeable with D_2O). MS m/z 285 [M$^+$]. Anal. Calcd for $C_{14}H_{15}N_5O_2$ (285.31): C, 58.94; H, 5.30; N, 24.55. Found: C, 58.91; H, 5.43; N, 24.85.

4.1.7. 2-Amino-4-(4-methylpiperazin-1-yl)-9*H*-pyrimido-[4,5-*b*]indol-6-ol (7f). Solvent A; eluent A, 7:3; 229 mg, 43%, green powder, mp >300 °C. IR (KBr): ν (cm^{-1}) = 2850, 1616, 1561, 1444, 1203, 617. ^{1}H NMR: (DMSO-d_6); δ = 2.29 (s, 3H, CH_3), 2.55 (m, 4H, H-3′, H-5′), 3.44 (m, 4H, H-2′, H-6′), 6.08 (s, 2H, NH_2, exchangeable with D_2O), 6.63 (dd, 1H, H-7, J_1 = 8.5 Hz, J_2 = 2.1 Hz), 6.87 (d, 1H, H-5, J = 2.1 Hz), 7.08 (d, 1H, H-8, J = 8.5 Hz), 8.91 (s, 1H, OH, exchangeable with D_2O), 10.95 (s, 1H, NH, exchangeable with D_2O). MS m/z 298 [M$^+$]. Anal. Calcd for $C_{15}H_{18}N_6O$ (298.35): C, 60.39; H, 6.08; N, 28.17. Found: C, 60.42; H, 5.89; N, 28.31.

4.1.8. 2-Amino-4-methoxy-9*H*-pyrimido[4,5-*b*]indol-6-ol (7g). Solvent A; eluent A, 273 mg, 30.8%, green powder, mp >300 °C. IR (KBr): ν (cm^{-1}) = 1609, 1511, 1383, 1239, 829. ^1H NMR: (DMSO-d_6); δ = 4.01 (s, 3H, $-CH_3$), 6.42 (s, 2H, NH_2, exchangeable with D_2O), 6.65 (dd, 1H, H-7, J_1 = 8.5 Hz, J_2 = 2.5 Hz), 7.10 (m, 2H, H-5, H-8), 8.85 (s, 1H, OH, exchangeable with D_2O), 11.04 (s, 1H, NH, exchangeable with D_2O). MS *m*/*z* 230 [M^+]. Anal. Calcd for $C_{11}H_{10}N_4O_2$ (230.23): C, 57.39; H, 4.38; N, 24.34. Found: C, 57.19; H, 4.33; N, 24.54.

4.1.9. 4-Amino-2-dimethylamino-9*H*-pyrimido[4,5-*b*]indol-6-ol (7h). Solvent A; eluent A, 203 mg, 27.6%, beige powder, mp 265–266 °C. IR (KBr): ν (cm^{-1}) = 2948, 1616, 1538, 1398, 1020. ^1H NMR: (DMSO-d_6); δ = 3.10 (s, 6H, NMe_2), 6.44 (s, 2H, NH_2, exchangeable with D_2O), 6.61 (dd, 1H, H-7, J_1 = 8.5 Hz, J_2 = 2.1 Hz), 6.98 (d, 1H, H-5, J = 2.1 Hz), 7.35 (d, 1H, H-8, J = 8.5 Hz), 8.63 (s, 1H, OH, exchangeable with D_2O), 10.86 (s, 1H, NH, exchangeable with D_2O). MS *m*/*z* 243 [M^+]. Anal. Calcd for $C_{12}H_{13}N_5O$ (243.27): C, 59.25; H, 5.31; N, 28.79. Found: C, 59.05; H, 5.37; N, 28.63.

4.1.10. 4-Amino-2-diethylamino-9*H*-pyrimido[4,5-*b*]indol-6-ol (7i). Solvent A; eluent A, 237 mg, 29.2%, gray plates, mp 235–236 °C. IR (KBr): ν (cm^{-1}) = 2963, 1592, 1554, 1401, 831. ^1H NMR: (DMSO-d_6); δ = 1.13 (t, 6H, J = 7.1 Hz, CH_3), 3.60 (q, 4H, J = 7.1 Hz, CH_2), 6.67 (s, 2H, NH_2, exchangeable with D_2O), 6.85 (dd, 1H, J_1 = 8.5 Hz, J_2 = 1.2 Hz, H-7), 7.16 (d, 1H, J = 8.5 Hz, H-8), 7.83 (d, 1H, J = 2.1 Hz, H-5), 8.83 (s, 1H, exchangeable with D_2O, OH), 11.29 (s, 1H, exchangeable with D_2O, NH). MS *m*/*z* 271 [M^+]. Anal. Calcd for $C_{14}H_{17}N_5O$ (271.32): C, 61.98; H, 6.32; N, 25.81. Found: C, 61.99; H, 6.42; N, 25.65.

4.1.11. 4-Amino-2-pyrrolidin-1-yl-9*H*-pyrimido[4,5-*b*]indol-6-ol (7j). Solvent A; eluent A, 204 mg, 25.3%, gray powder, mp >300 °C. IR (KBr): ν (cm^{-1}) = 3044, 2849, 1635, 1559, 1220, 1043. ^1H NMR: (DMSO-d_6); δ = 1.89 (m, 4H, H-3′, H-4′), 3.40 (m, 4H, H-2′, H-5′), 6.45 (s, 2H, NH_2, exchangeable with D_2O), 6.65 (dd, 1H, H-7, J_1 = 8.5 Hz, J_2 = 2.1 Hz), 6.95 (d, 1H, H-8, J = 8.5 Hz), 7.34 (d, 1H, H-5, J = 2.1 Hz), 8.79 (s, 1H, OH, exchangeable with D_2O), 10.90 (s, 1H, NH, exchangeable with D_2O). MS *m*/*z* 269 [M^+]. Anal. Calcd for $C_{14}H_{15}N_5O$ (269.32): C, 62.44; H, 5.61; N, 26.00. Found: C, 62.63; H, 5.34; N, 25.99.

4.1.12. 4-Amino-2-morpholin-4-yl-9*H*-pyrimido[4,5-*b*]indol-6-ol (7k). Solvent A; eluent A, 273 mg, 32%, brown powder, mp 260–261 °C. IR (KBr): ν (cm^{-1}) = 2854, 1614, 1419, 1268, 1112. ^1H NMR: (DMSO-d_6); δ = 3.58–3.72 (m, 8H, H-2′, H-3′, H-5′, H-6′), 6.65 (s, 2H, NH_2, exchangeable with D_2O), 6.63 (dd, 1H, H-7, J_1 = 8.5 Hz, J_2 = 2.5 Hz), 7.02 (d, 1H, H-5, J = 2.5 Hz), 7.39 (d, 1H, H-8, J = 8.5 Hz), 8.69 (s, 1H, OH, exchangeable with D_2O), 10.92 (s, 1H, NH, exchangeable with D_2O). MS *m*/*z* 285 [M^+]. Anal. Calcd for $C_{14}H_{15}N_5O_2$ (285.31): C, 58.94; H, 5.30; N, 24.55. Found: C, 59.11; H, 5.04; N, 24.76.

4.1.13. 4-Amino-2-(4-methylpiperazin-1-yl)-9*H*-pyrimido[4,5-*b*]indol-6-ol (7l). Solvent A; eluent A, 385 mg, 45%, green powder, mp >300 °C (EtOH). IR (KBr): ν (cm^{-1}) = 2848, 1610, 1504, 1201, 751. ^1H NMR: (DMSO-d_6); δ = 2.20 (s, 3H, CH_3), 2.33 (m, 4H, H-3′, H-5′), 3.71 (m, 4H, H-2′, H-6′), 6.53 (s, 2H, NH2, exchangeable with D_2O), 6.63 (dd, 1H, H-7, J_1 = 8.5 Hz, J_2 = 2.1 Hz), 7.00 (dd, 1H, H-8, J = 8.5 Hz), 7.83 (d, 1H, H-5, J = 2.1 Hz), 8.93 (s, 1H, OH, with D_2O exchangeable), 10.88 (s, 1H, NH, with D_2O exchangeable). MS *m*/*z* 298 [M^+]. Anal. Calcd for $C_{15}H_{18}N_6O$ (298.35): C, 60.39; H, 6.08; N, 28.17. Found: C, 60.18; H, 6.38; N, 28.43.

4.1.14. 2,4-Bis-(dimethylamino)-9*H*-pyrimido[4,5-*b*]indol-6-ol (7m). Solvent A; eluent A, 237 mg, 30%, brown powder, mp 202 °C (ethylacetate). IR (KBr): ν (cm^{-1}) = 2923, 2852, 1596, 1388, 1209. ^1H NMR: (DMSO-d_6); δ = 3.13 (s, 12H, CH_3), 6.61 (dd, 1H, J_1 = 2.1 Hz, J_2 = 8.5 Hz, H-7), 7.03 (d, 1H, J = 8.5 Hz, H-8), 7.07 (d, 1H, J = 2.1 Hz, H-5), 8.79 (s, 1H, exchangeable with D_2O, OH), 11.08 (s, 1H, exchangeable with D_2O, NH). MS *m*/*z* 271 [M^+]. Anal. Calcd for $C_{14}H_{17}N_5O$ (271.32): C, 61.98; H, 6.32; N, 25.81. Found: C, 61.83; H, 6.04; H, 25.76.

4.1.15. 2,4-Bis-(diethylamino)-9*H*-pyrimido[4,5-*b*]indol-6-ol (7n). Solvent A; eluent A, 31%, green powder, mp 218–219 °C (ethylacetate). IR (KBr): ν (cm^{-1}) = 2975, 1633, 1345, 753. ^1H NMR: (DMSO-d_6); δ = 1.21 (t, 6H, $-CH_2-CH_3$, J = 7.0 Hz), 1.33 (t, 6H, $-CH_2-CH_3$), J = 7.0 Hz, 3.66 (q, 4H, $-CH_2-CH_3$), J = 7 Hz, 3.80 (q, 4H, $-CH_2-CH_3$), J = 7 Hz, 6.75 (dd, H-7, J_1 = 8.5 Hz, J_2 = 2.1 Hz), 7.07 (d, 1H, H-5, J = 2.1 Hz), 7.44 (d, 1H, H-8, J = 8.5 Hz), 9.25 (s, 1H, OH, exchangeable with D_2O), 11.35 (s, 1H, NH, exchangeable with D_2O). MS *m*/*z* 327 [M^+]. Anal. Calcd for $C_{18}H_{25}N_5O$ (327.42): C, 66.03; H, 7.70; N, 21.39. Found: C, 66.28; H, 7.94; H, 21.56.

4.1.16. 2,4-Dipyrrolidin-1-yl-9*H*-pyrimido[4,5-*b*]indol-6-ol (7o). Solvent A; eluent A, The residue was crystallized from EtOH after cooling and evaporation. 532 mg, 54.9%, green powder, mp 221–222 °C. IR (KBr): ν (cm^{-1}) = 2975, 2881, 1643, 1452, 1213, 655. ^1H NMR: (DMSO-d_6); δ = 1.91 (m, 8H, H-3′, H-5′, H-3″, H-4″) 3.50–3.79 (m, 8H, H-2′, H-5′, H-3″, H-4″), 6.57 (dd, 1H, H-7, J_1 = 8.5 Hz, J_2 = 2.1 Hz), 6.97 (d, 1H, H-8, J = 8.5 Hz), 7.27 (d, 1H, H-5, J = 2.1 Hz), 8.68 (s, 1H, OH, exchangeable with D_2O), 11.05 (s, 1H, NH, exchangeable with D_2O). MS *m*/*z* 323 [M^+]. Anal. Calcd for $C_{18}H_{21}N_5O$ (323.40): C, 66.85; H, 6.55; N, 21.66. Found: C, 66.91; H, 6.37; N, 21.69.

4.1.17. 2,4-Dimorpholin-4-yl-9*H*-pyrimido[4,5-*b*]indol-6-ol (7p). Solvent A; eluent A, 323 mg, 30.4%, tan powder, mp 169–170 °C. IR (KBr): ν (cm^{-1}) = 3008, 2854, 1600, 1554, 1257, 1195, 1112, 754. ^1H NMR: (DMSO-d_6); δ = 3.49–3.93 (m, 16H, H-2′, H-3′, H-5′, H-6′, H-2″, H-3″, H5″, H6″), 6.68 (dd, 1H, H-7, J_1 = 8.5 Hz, J_2 = 2.1 Hz), 6.88 (d, 1H, H-5, J = 2.1 Hz), 7.10 (d, 1H, H-8, J = 8.5 Hz), 8.96 (s, 1H, OH, exchangeable with D_2O), 11.23 (s, 1H, NH, exchangeable with

D_2O). MS *m/z* 355 [M^+]. Anal. Calcd for $C_{18}H_{21}N_5O_3$ (355.40): C, 60.83; H, 5.96; N, 19.71. Found: C, 60.98; H, 6.23; N, 19.62.

4.1.18. 2,4-Bis-(4-methylpiperazin-1-yl)-9*H*-pyrimido[4,5-*b*]indol-6-ol (7q). Solvent A; eluent A, 7:3; 406 mg, 35.5%, green powder, mp 225–228 °C. IR (KBr): *v* (cm^{-1}) = 2846, 2802, 1600, 1552, 1446, 1197, 1002. ^1H NMR: (DMSO-d_6); δ = 2.21 (s, 1H, CH_3), 2.28 (s, 1H, CH_3), 2.35–2.55 (m, 8H, H-3′, H-5′, H-3″, H-5″), 2.35–2.55 (m, 8H, H-3′, H-5′, H-3″, H-5″), 3.50–3.74 (m, 8H, H-2′, H-6′, H-2″, H-6″), 6.67 (dd, 1H, H-7, J_1 = 8.5 Hz, J_2 = 2.1 Hz), 6.89 (d, 1H, H-5, *J* = 2.1 Hz), 7.08 (d, 1H, H-8, *J* = 8.5 Hz), 9.02 (s, 1H, OH, exchangeable with D_2O), 11.15 (s, 1H, NH, exchangeable with D_2O). MS *m/z* 381 [M^+]. Anal. Calcd for $C_{20}H_{27}N_7O$ (381.48): C, 62.97; H, 7.13; N, 25.70. Found: C, 62.98; H, 7.08; N, 25.50.

4.1.19. 7,9-Diamino-11*H*-benzo[*g*]pyrimido[4,5-*b*]indol-5-ol (14a). Solvent A; eluent A, the product **14a** deposits from the hot reaction mixture and is washed with EtOH/H_2O; 375 mg, 38.1%, deep blue powder, mp >300 °C. IR (KBr): *v* (cm^{-1}) = 2415, 1761, 1671, 1383, 1217, 756. ^1H NMR: (DMSO-d_6); δ = 6.00 (s, 2H, NH_2, exchangeable with D_2O), 6.83 (s, 2H, NH_2, exchangeable with D_2O), 7.46 (td, 1H, H-8, J_1 = 8.0 Hz, J_2 = 1.0 Hz), 7.48 (t, 1H, H-9, J_1 = 8.0 Hz, J_2 = 1.0 Hz), 7.86 (d, 1H, H-7, *J* = 8.0 Hz), 8.14 (s, 1H, H-5), 8.44 (d, 1H, H-10, *J* = 8.0 Hz), 10.44 (s, 1H, OH, exchangeable with D_2O), 12.15 (s, 1H, NH, exchangeable with D_2O). MS *m/z* 265 [M^+]. Anal. Calcd for $C_{14}H_{11}N_5O$ (265.28): C, 63.39; H, 4.18; N, 26.40. Found: C, 63.22; H, 4.46; N, 26.69.

4.1.20. 7-Amino-9-(morpholin-4-yl)-11*H*-benzo[*g*]pyrimido[4,5-*b*]indol-5-ol (14b). The educts are stirred at 100 °C in HOAc. After evaporation in vacuo, the residue is crystallized from EtOH. 568 mg, 56.2%, tan powder, mp >300 °C. IR (KBr): *v* (cm-1) = 3420, 1637, 1535, 1344, 1072. ^1H NMR: (DMSO-d_6); δ = 3.72–3.86 (m, 8H, H-2′, H-3′, H-5′, H-6′), 6.83 (s, 2H, NH_2, exchangeable with D_2O), 7.47 (s, 1H, H-5), 7.56 (td, 1H, H-8, J_1 = 8.2 Hz, J_2 = 1.0 Hz), 7.65 (td, 1H, H-9, *J* = 8.2 Hz, J_2 = 1.0 Hz), 7.90 (d, 1H, H-7, *J* = 8.2 Hz), 8.53 (d, 1H, H-10, *J* = 8.2 Hz), 10.48 (s, 1H, OH, exchangeable with D_2O), 12.73 (s, 1H, NH, exchangeable with D_2O). MS *m/z* 335 [M^+]. Anal. Calcd for $C_{18}H_{17}N_5O_2$: C, 64.47; H, 5.11; N, 20.88. Found: C, 64.69; H, 5.33; N, 20.61.

4.1.21. 7-Amino-9-(4-methylpiperazin-1-yl)-11*H*-benzo[*g*]-pyrimido[4,5-*b*]indol-5-ol (14c). Solvent A; eluent A, 187 mg, 18.1%, dark gray powder, mp >300 °C. IR (KBr): *v* (cm^{-1}) = 3409, 1611, 1533, 1344, 1041, 761. ^1H NMR: (DMSO-d_6); δ = 2.38 (s, 3H, CH_3), 2.61–2,73 (m, 4H, H-3′, H-5′) 3.63–3.79 (m, 4H, H-2′, H-6′), 6.92 (s, 2H, NH_2, exchangeable with D_2O), 7.59 (td, 1H, H-8, J_1 = 8.2 Hz, J_2 = 1.0 Hz), 7.69 (td, 1H, H-9, J_1 = 8.2 Hz, J_2 = 1.0 Hz), 7.92 (d, 1H, H-7, *J* = 8.2 Hz), 8.31 (s, 1H, H-5), 8.47 (d, 1H, H-10, *J* = 8.2 Hz), 10.16 (s, 1H, OH, exchangeable with D_2O), 12.34 (s, 1H, NH, exchangeable with D_2O). MS *m/z* 348 [M^+]. Anal. Calcd for $C_{19}H_{20}N_6O$ (348.41): C, 65.50; H, 5.79; N, 24.12. Found: C, 65.34; H, 5.63; N, 24.41.

4.1.22. 7-Amino-9-pyrrolidin-1-yl-11H-benzo[*g*]pyrimido[4,5-*b*]indol-5-ol (14d). Solvent A; eluent A, 165 mg, 17.2%, gray powder, mp >300 °C. IR (KBr): *v* (cm^{-1}) = 3411, 1633, 1616, 1529, 1344, 1068, 765. ^1H NMR: (DMSO-d_6); δ = 1.88 (m, 4H, H-3′, H-4′), 3.3.2 (m, 4H, H-2′, H-5′), 5.93 (s, 2H, with D_2O exchangeable, NH_2), 7.44 (t, J_1 = 1.0 Hz, J_2 = 8.2 Hz, H-8), 7.58 (td, 1H, J_1 = 1.0, J_2 = 8.2 Hz, H-9), 8.1 (s, 1H, H-5), 8.24 (d, 1H, *J* = 8.2 Hz, H-7), 8.59 (d, 1H, *J* = 8.2 Hz, H-7), 10.19 (s, OH, with D_2O exchangeable), 12.22 (s, 1H, NH, with D_2O exchangeable). MS *m/z* 319 [M^+]. Anal. Calcd for $C_{18}H_{17}N_5O$ (319.37): C, 67.70; H, 5.37; N, 21.93. Found: C, 67.40; H, 5.65; N, 21.67.

4.1.23. 9-Amino-7-(morpholin-4-yl)-11*H*-benzo[*g*]pyrimido[4,5-*b*]indol-5-ol (14e). Solvent A; eluent A, 95:5. 275 mg, 27.4%, gray powder, mp >300 °C. IR (KBr): *v* (cm^{-1}) = 3747, 2921, 2337, 1733, 1589, 800. ^1H NMR: (DMSO-d_6); δ = 3.70–3.85 (m, 8H, H-2′, H-3′, H-5′, H-6′), 6.03 (s, 2H, NH_2, exchangeable with D_2O), 7.45 (s, 1H, H-5), 7.57 (td, 1H, H-8, J_1 = 8.2 Hz, J_2 = 1.0 Hz), 7.63 (td, 1H, H-9, *J* = 8.2 Hz, J_2 = 1.0 Hz), 7.91 (d, 1H, H-7, *J* = 8.2 Hz), 8.51 (d, 1H, H-10, *J* = 8.2 Hz), 10.45 (s, 1H, OH, exchangeable with D_2O), 12.71 (s, 1H, NH, exchangeable with D_2O). MS *m/z* 335 [M^+]. Anal. Calcd for $C_{18}H_{17}N_5O_2$ (335.37): C, 64.47; H, 5.11; N, 20.88. Found: C, 64.33; H, 5.00; N, 20.97.

4.1.24. 7-Amino-9-pyrrolidin-1-yl-11*H*-pyrimido[5′,4′:4,5]-pyrrolo[2,3-*f*]quinolin-5-ol (16). Solvent B; eluent A, 96 mg, 10.6%, green powder, mp >300 °C. IR (KBr): *v* (cm^{-1}) = 2923, 2351, 1756, 899. ^1H NMR: (DMSO-d_6); δ = 1.98 (m, 4H, H-3′, H-4′), 3.56 (m, 4H, H-2′, H-5′), 6.89 (s, 2H, NH_2, exchangeable with D_2O), 7.60 (dd, 1H, H-9, J_1 = 8.5 Hz, J_2 = 5.7 Hz), 7.80 (s, 1H, H-5), 8.74 (d, 1H, H-8, *J* = 8.5 Hz), 8.85 (d, 1H, H-10, *J* = 5.7 Hz), 9.16 (s, 1H, OH, exchangeable with D_2O), 12.55 (s, 1H, NH, exchangeable with D_2O). MS *m/z* 320 [M^+]. Anal. Calcd for $C_{17}H_{16}N_6O$ (320.36): C, 63.74; H, 5.03; N, 26.23. Found: C, 63.99; H, 5.00; N, 25.97.

4.1.25. 7-Amino-9-pyrrolidin-1-yl-11*H*-pyrimido[5′,4′:4,5]-pyrrolo[2,3-*f*]quinoxalin-5-ol (18). Solvent B; eluent A, 182 mg, 19%, green powder, mp >300 °C. IR (KBr): *v* (cm^{-1}) = 2915, 1665, 1556, 1171, 1029. ^1H NMR: (DMSO-d_6); δ = 1.92 (m, 4H, H-3′, H-4′), 3.53 (m, 4H, H-2′, H-5′), 6.76 (s, 2H, NH_2, exchangeable with D_2O), 7.99 (s, 1H, H-6), 8.76 (d, 1H, *J* = 1.8 Hz), 8.87 (d, 1H, *J* = 1.8 Hz), 9.41 (s, 1H, OH, exchangeable with D_2O), 12.02 (s, 1H, NH, exchangeable with D_2O). MS *m/z* 321 [M^+]. Anal. Calcd for $C_{16}H_{15}N_7O$ (321.34): C, 59.80; H, 4.71; N, 30.51. Found: C, 59.97; H, 5.00; N, 30.29.

4.1.26. 6-Methoxy-2-pyrrolidin-1-yl-9*H*-pyrimido[4,5-*b*]-indol-4-amine (19). Compound **7j** (100 mg, 0.36 mmol) and 14 mg of a NaH dispersion (60%) are stirred in

acetone (25 ml) for 15 min. After evolution of hydrogen gas stopped, 100 mg methyliode (0.72 mmol) was added and the mixture was stirred for 12 h. The acetone was distilled off in vacuo and the resulting residue was taken up in 2 N NaOH (20 ml) and was extracted three times with ethylacetate. After evaporation, a gray powder (36 mg) was isolated. Yield: 34%, mp >300 °C. IR (KBr): ν (cm^{-1}) = 3044, 2849, 1635, 1559, 1220, 1043. ^1H NMR: (DMSO-d_6); δ = 1.83 (m, 4H, H-3′, H-4′) 3.51 (m, 4H, H-2′, H-5′), 3.88 (s, 3H, CH_3), 6.63 (s, 2H, NH_2, exchangeable with D_2O), 6.65 (dd, 1H, H-7 J_1 = 8.5 Hz, J_2 = 2.1 Hz), 7.03 (d, 1H, H-8, J = 8.5 Hz), 7.63 (d, 1H, H-5, J = 2.1 Hz), 11.2 (s, 1H, NH, exchangeable with D_2O). MS m/z 283 [M^+]. Anal. Calcd for $C_{15}H_{17}N_5O$ (283.34): C, 63.59; H, 6.05; N, 24.72. Found: C, 63.71; H, 6.30; N, 24.87.

4.1.27. 2-Pyrrolidin-1-yl-5,6,7,8-tetrahydro-9*H*-pyrimido[4,5-*b*]indol-4-amine (21). 2-Pyrrolidin-4-ylpyrimidin-4,6-diamine (**9j**) (179 mg, 1 mmol) and 2-chlorocyclohexanone (**20**) (132 mg, 1 mmol) were heated to reflux for 6 h. After evaporation in vacuo, the residue was purified by MPLC. Eluent A, 113 mg, 43.9%, colorless powder, mp 260–261 °C. IR (KBr): ν (cm^{-1}) = 2923, 2362, 1735, 1614, 1068. ^1H NMR: (DMSO-d_6); δ = 1.72 (m, 4H, H-6, H-7), 1.86 (m, 4H, H-3′, H-4′) 2.46–2.65 (m, 4H, H-5, H-8), 3.40 (m, 4H, H-2′, H-5′), 5.45 (s, 2H, NH_2, exchangeable with D_2O), 10.45 (s, 1H, NH, exchangeable with D_2O). MS m/z 257 [M^+]. Anal. Calcd for $C_{14}H_{19}N_5$ (257.36): C, 65.34; H, 7.44; N, 27.21. Found: C, 65.39; H, 7.21; N, 27.23.

4.1.28. 2-Pyrrolidin-1-yl-9*H*-pyrimido[4,5-*b*]indol-4-amine (22). Compound **21** (101 mg, 0.4 mmol) and palladium/charcoal catalyst 5% (10 mg) are suspended in xylene (150 ml) and heated to reflux for 8 h. After filtration and evaporation in vacuo the residue was purified by MPLC. Eluent A, 41 mg, 25.3%, tan powder, mp 281–283 °C. IR (KBr): ν (cm^{-1}) = 2950, 2867, 1610, 1517, 1413, 1226. ^1H NMR: (DMSO-d_6); δ = 1.90 (m, 4H, H-3′, H-4′), 3.50 (m, 4H, H-2′, H-5′), 6.45 (s, 2H, NH_2, exchangeable with D_2O), 7.03 (t, 1H, H-6, J = 7.5 Hz), 7.05 (t, 1H, H-7, J = 7.5 Hz), 7.19 (d, 1H, H-6, J = 7.5 Hz), 7.98 (d, 1H, H-8, J = 7.5 Hz), 11.26 (s, 1H, NH, exchangeable with D_2O). MS m/z 253 [M^+]. Anal. Calcd for $C_{14}H_{15}N_5$ (253.31): C, 66.38; H, 5.97; N, 27.65. Found: C, 66.36; H, 6.11; N, 27.84.

4.1.29. 4-Amino-9*H*-pyrimido[4,5-*b*]indol-6-ol (24). Compound **23** (100 mg, 0.58 mmol), 1 ml formamide, 0.3 ml dimethylformamide, and 0.15 ml of formic acid (85%) were refluxed over a period of 2 h. After cooling to rt, the surplus reagents were distilled off in vacuo and the resulting residue was purified via MPLC. Solvent: cyclohexane/ethylacetate 6:4, 30 mg, 27%, gray powder, mp >300 °C. Crystallized from cyclohexane/ethylacetate. IR (KBr): ν (cm^{-1}) = 3740, 3690, 3650, 3630, 3310, 1650, 1625, 1560, 1540, 1508, 1295, 1180, 915, 795, 670, 650. ^1H NMR: (DMSO-d_6); δ = 6.86 (dd, H, J_1 = 2.5, J_2 = 8.5 Hz, H-7), 6.92 (s, 2H, exchangeable with D_2O, NH_2), 7.22 (d, 1H, J = 8.5 Hz, H-8), 7.61 (d, 1H, J = 2.5 Hz, H-5), 8.17 (s, 1H, H-2), 8.90 (s, 1H, exchangeable with D_2O, OH), 11.44 (s, 1H, exchangeable with D_2O, NH). MS m/z 200 [M^+]. Anal. Calcd for $C_{10}H_8N_4O$ (200.2): C, 60.00; H, 4.03; N, 27.99. Found: C, 59.68; H, 4.05; N, 27.90.

4.1.30. 4-Phenyl-9*H*-pyrimido[4,5-*b*]indol-6-ol (26). Compound **25** (200 mg, 0.79 mmol) and 300 mg formic acid (85%) in 2 ml formamide were refluxed over a period of 30 min. After cooling to rt, the surplus reagents was evaporated in vacuo and the resulting residue was purified via MPLC. Solvent: cyclohexane/ethylacetate 7:3, 124 mg, 60%, yellow crystals, mp >300 °C. Crystallized from cyclohexane/ethylacetate. IR (KBr): ν (cm^{-1}) = 3550, 3480, 3415, 3080, 2815, 2680, 1685, 1590, 1565, 1420, 1360, 1320, 1275, 1200, 1155, 965, 930. ^1H NMR: (DMSO-d_6); δ = 7.00 (dd, 1H, J_1 = 2.5 Hz, J_2 = 8.5 Hz, H-7), 7.20 (d, 1H, J = 2.5 Hz, H-5), 7.40 (d, 1H, J = 8.5 Hz, H-8), 7.64 (m, 3H, H-3′, H-4′, H-5′), 7.83 (m, 2H, H-2′, H-6′), 8.90 (s, 1H, H-2), 9.18 (s, 1H, exchangeable with D_2O, OH), 12.18 (s, 1H, exchangeable with D_2O, NH). MS m/z 261 [64, M^+], Anal. Calcd for $C_{16}H_{11}N_3O$ (261.3): C, 73.55; H, 4.24; N, 16.08. Found: C, 73.31; H, 4.25; N, 16.27.

4.2. Cell proliferation assay

Details of the cytotoxic testing and calculation of the IC_{50} values have been described in detail elsewhere.[16,17] All compounds were dissolved in DMSO (cell culture grade) to give stock solutions of 20 mM and stored at −20 °C until used. On the day of testing, the stock solutions were thawed and serially diluted in DMSO to the desired working concentration range (i.e., 20, 10, 5.0, 2.50, 1.25, and 0.67 mM). Depending on the expected potency, five working solutions were selected and diluted 1000-fold into cell culture medium to treat the cells. The actively dividing cells were exposed to test substances for a total of 96 h before fixing with glutaraldehyde and staining with crystal violet.

4.3. QSAR-methods

All molecules were first geometry-optimized using the semi-empirical program package VAMP[19] applying the AM1[20] hamiltonian. Rotatable substituents attached to the aromatic scaffold in positions 2 and 4 were systematically scanned applying VAMP's built-in TORQUE function, ensuring to choose the minimum-energy conformation reflecting the absolute minimum in the gas phase. Due to the basic character of the aromatic nitrogens present in every compound, all molecules were considered to be positively charged. The location of the protonation at N1 was determined by comparing the heat of formation of the different mono-protonated isomers and choosing the energetically most favorable. Partial atomic charges were calculated using the Vamp ElectroStatic Potential fit Approach VESPA.[21] The calculation of the molecular descriptors and all data analysis were performed using the molecular spreadsheet TSAR.[22] The initially generated descriptors range from molecular attributes (mass, surface area, volume, Verloop sterical parameters, moments of inertia, dipole moments, molar refractivity, lipophilicity, and lipole), indices (connectivity, shape,

and topology), to atom, ring, and group counts. Additionally, the semi-empirical geometry optimization provided quantum-mechanical descriptors like heat of formation, HOMO and LUMO energies, and partial charges on atoms present in all molecules of the data set. As target for the activity prediction by multiple linear regression, the average of the four in vitro tests 5637, RT-4, A-427, and LCLC-103H, represented as $\log 1/IC_{50}$, was used. Variable reduction and selection was done by calculating correlation matrices and careful automatic F stepping, followed by manual removing of single descriptors. The quality of the model was checked throughout the data reduction procedure by calculating cross-validated regression coefficients (r^2_{CV}).

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2006.06.051.

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